Claims:

1. A method for preparing a nucleic acid binding protein of the Cys2-His2 zinc finger class capable of binding to a nucleic acid quadruplet in a target nucleic acid sequence, wherein binding to base 4 of the quadruplet by an α-helical zinc finger nucleic acid binding motif in the protein is determined as follows:

a) if base 4 in the quadruplet is A, then position +6 in the α-helix is Glu, Asn or Val;
b) if base 4 in the quadruplet is C, then position +6 in the α-helix is Ser, Thr, Val, Ala, Glu or Asn.

2. A method according to claim 1, wherein binding to base 4 of the quadruplet by an α-helical zinc finger nucleic acid binding motif in the protein is additionally determined as follows:

c) if base 4 in the quadruplet is G, then position +6 in the α-helix is Arg or Lys;
d) if base 4 in the quadruplet is T, then position +6 in the α-helix is Ser, Thr, Val or Lys.

3. A method for preparing a nucleic acid binding protein of the Cys2-His2 zinc finger class capable of binding to a nucleic acid quadruplet in a target nucleic acid sequence, wherein binding to each base of the quadruplet by an α-helical zinc finger nucleic acid binding motif in the protein is determined as follows:

a) if base 4 in the quadruplet is G, then position +6 in the α-helix is Arg or Lys;
b) if base 4 in the quadruplet is A, then position +6 in the α-helix is Glu, Asn or Val;
c) if base 4 in the quadruplet is T, then position +6 in the α-helix is Ser, Thr, Val or Lys;
d) if base 4 in the quadruplet is C, then position +6 in the α-helix is Ser, Thr, Val, Ala, Glu or Asn;
e) if base 3 in the quadruplet is G, then position +3 in the α-helix is His;
f) if base 3 in the quadruplet is A, then position +3 in the α-helix is Asn;

g) if base 3 in the quadruplet is T, then position +3 in the α-helix is Ala, Ser or Val; provided that if it is Ala, then one of the residues at -1 or +6 is a small residue;

h) if base 3 in the quadruplet is C, then position +3 in the α-helix is Ser, Asp, Glu, Leu, Thr or Val;

i) if base 2 in the quadruplet is G, then position -1 in the α-helix is Arg;

j) if base 2 in the quadruplet is A, then position -1 in the α-helix is Gln;

k) if base 2 in the quadruplet is T, then position -1 in the α-helix is His or Thr;

l) if base 2 in the quadruplet is C, then position -1 in the α-helix is Asp or His.

m) if base 1 in the quadruplet is G, then position +2 is Glu;

n) if base 1 in the quadruplet is A, then position +2 Arg or Gln;

o) if base 1 in the quadruplet is C, then position +2 is Asn, Gln, Arg, His or Lys;

p) if base 1 in the quadruplet is T, then position +2 is Ser or Thr.

4. A method according to any preceding claim, wherein the or each zinc finger has the general primary structure of SEQ ID NO:3

(A) X^a C X_{2-4} C X_{2-3} F X^c X X X X L X X H X X X^b H - linker

-1 1 2 3 4 5 6 7 8 9

wherein X (including X^a, X^b and X^c) is any amino acid.

Sub E'

5. A method according to claim 5 wherein X^a is $^F/_Y$-X or P-$^F/_Y$-X.

6. A method according to claim 4 or claim 5 wherein X_{2-4} is selected from any one of: S-X, E-X, K-X, T-X, P-X and R-X.

7. A method according to any one of claims 4 to 6 wherein X^b is T or I.

8. A method according to any one of claims 4 to 7 wherein X_{2-3} is G-K-A, G-K-C, G-K-S, G-K-G, M-R-N or M-R.

9. A method according to any one of claims 4 to 8 wherein the linker is T-G-E-K or T-G-E-K-P.

10. A method according to any one of claims 4 to 9 wherein position +9 is R or K.

Sub E' cont

11. A method according to any one of claims 4 to 10 wherein positions +1, +5 and +8 are not occupied by any one of the hydrophobic amino acids, F, W or Y.

12. A method according to claim 11 wherein positions +1, +5 and +8 are occupied by the residues K, T and Q respectively.

Sub E2

13. A method for preparing a nucleic acid binding protein of the Cys2-His2 zinc finger class capable of binding to a target nucleic acid sequence, comprising the steps of:

a) selecting a model zinc finger domain from the group consisting of naturally occurring zinc fingers and consensus zinc fingers; and

b) mutating the finger according to the rules set in any one of claims 1 to 3.

Sub B12

14. A method according to claim 13, wherein the model zinc finger is a consensus zinc finger whose structure is selected from the group consisting of the consensus structure of SEQ ID NO:5 P Y K C P E C G K S F S Q K S D L V K H Q R T H T G, and the consensus structure of SEQ ID NO:6 P Y K C S E C G K A F S Q K S N L T R H Q R I H T G E K P.

15. A method according to claim 13 wherein the model zinc finger is a naturally occurring zinc finger whose structure is selected from one finger of a protein selected from the group consisting of Zif 268 (Elrod-Erickson *et al.*, (1996) Structure 4:1171-1180), GLI (Pavletich and Pabo, (1993) Science 261:1701-1707), Tramtrack (Fairall *et al.*, (1993) Nature 366:483-487) and YY1 (Houbaviy *et al.*, (1996) PNAS (USA) 93:13577-13582).

16. A method according to claim 15 wherein the model zinc finger is finger 2 of Zif 268.

Sub E3

17. A method according to any preceding claim wherein the binding protein comprises two or more zinc finger binding motifs, placed N-terminus to C-terminus.

Sub D13

18. A method according to claim 14, wherein the N-terminal zinc finger is preceded by a leader peptide having the sequence MAEEKP.

Sub E4

19. A method according to claim 14 or claim 15, wherein the nucleic acid binding protein is constructed by recombinant nucleic acid technology, the method comprising the steps of:

a) preparing a nucleic acid coding sequence encoding two or more zinc finger binding motifs as defined in any one of claims 5 to 13, placed N-terminus to C-terminus;
b) inserting the nucleic acid sequence into a suitable expression vector; and
c) expressing the nucleic acid sequence in a host organism in order to obtain the nucleic acid binding protein.

20. A method according to any preceding claim comprising the additional steps of subjecting the nucleic acid binding protein to one or more rounds of randomisation and selection in order to improve the characteristics thereof.

21. A method according to claim 20, wherein the randomisation and selection is carried out by phage display technology.

22. A method according to claim 21, comprising the steps of:

a) preparing a nucleic acid construct capable of expressing a fusion protein comprising the nucleic acid binding protein and a minor coat protein of a filamentous bacteriophage;

b) preparing further nucleic acid constructs capable of expressing a fusion protein comprising a selectively mutated nucleic acid binding protein and a minor coat protein of a filamentous bacteriophage;

c) causing the fusion proteins defined in steps (a) and (b) to be expressed on the surface of bacteriophage transformed with the nucleic acid constructs;

d) assaying the ability of the bacteriophage to bind the target nucleic acid sequence and selecting the bacteriophage demonstrating superior binding characteristics.

Sub E5

23. A method according to any one of claims 20 to 22 wherein the nucleic acid binding protein is selectively randomised at any one of positions +1, +5, +8, -1, +2, +3 or +6.

24. A method according to claim 23, wherein, in the nucleic acid binding protein, position +6 of a zinc finger and positions -1, +1, +2 and +3 of an adjacent zinc finger are randomised.

Sub E6

25. A method for determining the presence of a target nucleic acid molecule, comprising the steps of:

a) preparing a nucleic acid binding protein by the method of any preceding claim which is specific for the target nucleic acid molecule;
b) exposing a test system comprising the target nucleic acid molecule to the nucleic acid binding protein under conditions which promote binding, and removing any nucleic acid binding protein which remains unbound;
c) detecting the presence of the nucleic acid binding protein in the test system.

26. A method according to claim 25, wherein the presence of the nucleic acid binding protein in the test system is detected by means of an antibody.

Sub E7

27. A method according to claim 25 or claim 26 wherein the nucleic acid binding protein, in use, is displayed on the surface of a filamentous bacteriophage and the presence

of the nucleic acid binding protein is detected by detecting the bacteriophage or a component thereof.

Sub E7 cont

28. A synthetic nucleic acid binding protein whose design incorporates a method according to any one of claims 1 to 24.

29. A nucleic acid encoding a nucleic acid binding protein according to claim 28.

30. A host cell transformed with a nucleic acid according to claim 29.

31. Use of a nucleic acid binding protein according to claim 28 or a nucleic acid according to claim 29 in medicine.

Add E10